Supplementary Information for

Tuning the Color Palette of Fluorescent Copper Sensors through Systematic Heteroatom Substitution at Rhodol Cores

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Spectroscopic methods and materials

All spectroscopic measurements were performed at 25 °C in 25 mM HEPES buffer (pH 7.4) prepared with Millipore water unless noted. Absorption spectra were recorded using a Varian Cary 60 spectrophotometer and fluorescence spectra were recorded using a Photon Technology International Quanta Master 4 L-format scan spectrofluorometer equipped with an LPS-220B 75-W xenon lamp, A-1010B lamp housing with integrated igniter, switchable 814 photocounting/analog photomultiplier detection unit and MD5020 motor driver. Samples for absorption and emission measurements were contained in 0.35 × 1 cm quartz cuvettes (1.4-mL volume; Starna). Excitation was provided at 580 nm, 616 nm and 650 nm for CCF1/Ctrl-CCF1, CSF1/Ctrl-CSF1 and CPF1/Ctrl-CPF1, respectively. Stock solutions of $[Cu(CH_3CN)_4]PF_6$ in acetonitrile were used to provide Cu^+. Metals used in the selectivity assays were derived from their chloride salts. The binding affinities of CCF1, CSF1 and CPF1 to Cu^+ were measured using thiourea as a competitive ligand to provide a buffered Cu^+ solution ($\beta_{12} = 2.0 \times 10^{12}$, $\beta_{13} = 2.0 \times 10^{14}$, $\beta_{14} = 3.4 \times 10^{15}$).[1] For characterization of probe responses at different pH values, buffers were prepared by neutralizing the free acid solutions of 25 mM HEPES, 25 mM MES and 25 mM acetic acid with 5 M NaOH. HEK 293T cell lysates were prepared in HEPES buffer with pump-freeze-thaw cycles and handled under a nitrogen atmosphere, with their concentrations adjusted to 1 mg/mL protein as analyzed by the Bradford assay.

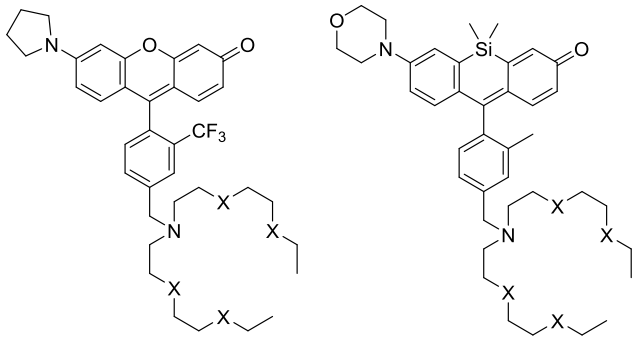
Image analysis and quantification

ImageJ (National Institutes of Health) was used for image analysis. For quantification of fluorescence intensity, each image was set to 8-bit greyscale and inverted. The fluorescence intensity was estimated using non-calibrated OD function. The area of stained cells was selected by setting appropriate threshold (≥0.051 for CCF1/Ctrl-CCF1, CSF1/Ctrl-CSF1, CPF1/Ctrl-CPF1 images and ≥0.032 for Calcium Green-1 images). The statistics of the image was then measured by the "Measure" function and the average fluorescence intensity was obtained by dividing the integrated density (IntDen) over area. For each condition, four images of different fields of cells from each biological replicate were analyzed using this process and the values were combined for statistical analysis.

Cell fractionation and Inductively Coupled Plasma (ICP)-MS analysis

$Atp7a^{-/-}$ and matched control MEFs were fractionated using the NE-PER kit (Thermo Fisher). Extracts were digested by adding equal volumes of concentrated nitric acid, incubated overnight at room temperature on a rotator, and boiled for 2 hours at 95 °C. Digested extracts were diluted into 2% nitric acid with an internal standard and run on an iCAP-Q ICP-MS in KED mode. The level of each element found in the extraction reagent was subtracted from each measurement. The resulting values were normalized to protein concentration.

Supplementary figures



Scheme S1. Chemical structures of previously reported copper sensors, CF3 and CSR1, along with their control analogs, Ctrl-CF3 and Ctrl-CSR1.

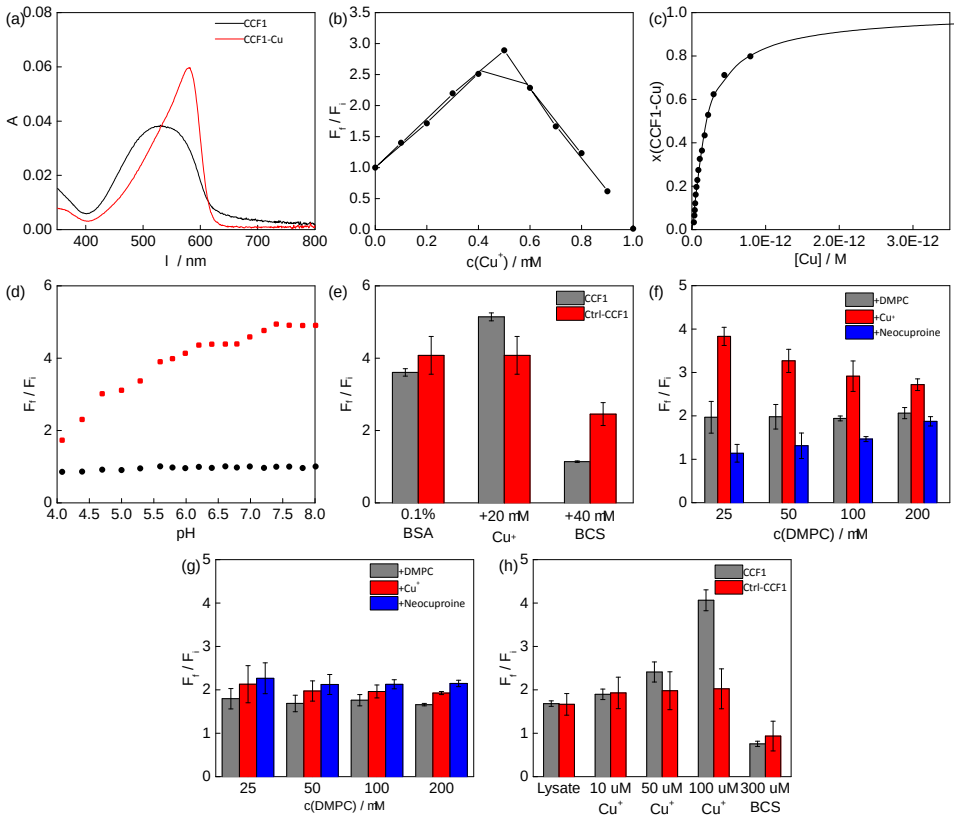


Figure S1. *In vitro* characterization of CCF1 and Ctrl-CCF1. For bar graphs, bars represent the final integrated fluorescence response (F_f) over the initial integrated emission (F_i) of CCF1 or Ctrl-CCF1 over 590–670 nm; values are shown as average ± s.d. (n=3). (a) UV-visible spectral change of 3 μM CCF1 (black) upon addition of 3 μM Cu^+ (red). (b) Job's plot of CCF1 and Cu^+. The total concentrations of CCF1 and Cu^+ were kept at 1 μM. (c) Fluorescence response of 1 μM CCF1 to thiourea-buffered Cu^+ solutions for the K_d measurement. The observed K_d value is 2.0×10^{-13} M. Solid line represents the calculated curve. (d) Fluorescence intensity of 1 μM CCF1 (black) and 1 μM CCF1-Cu (red) across a range of pH values. (e) Fluorescence response of 1 μM CCF1 (grey) or Ctrl-CCF1 (red) to 0.1% BSA and subsequent addition of 20 μM Cu^+ and 40 μM BCS. (f) Fluorescence response of 1 μM CCF1 in the presence of DMPC, a lipid-forming reagent (grey), with subsequent addition of 1 μM Cu^+ to the solution (red), and a final addition of 10 μM neocuproine (blue). (g) Fluorescence response of 1 μM Ctrl-CCF1 in the presence of DMPC (grey), with subsequent addition of 1 μM Cu^+ to the solution (red), and a final addition of 10 μM neocuproine (blue). (h) Fluorescence response of 1 μM CCF1 (grey) and Ctrl-CCF1 (red) to HEK 293T lysates (1 mg protein/mL) and subsequent addition of 10, 50 and 100 μM Cu^+, followed by addition of 300 μM BCS.

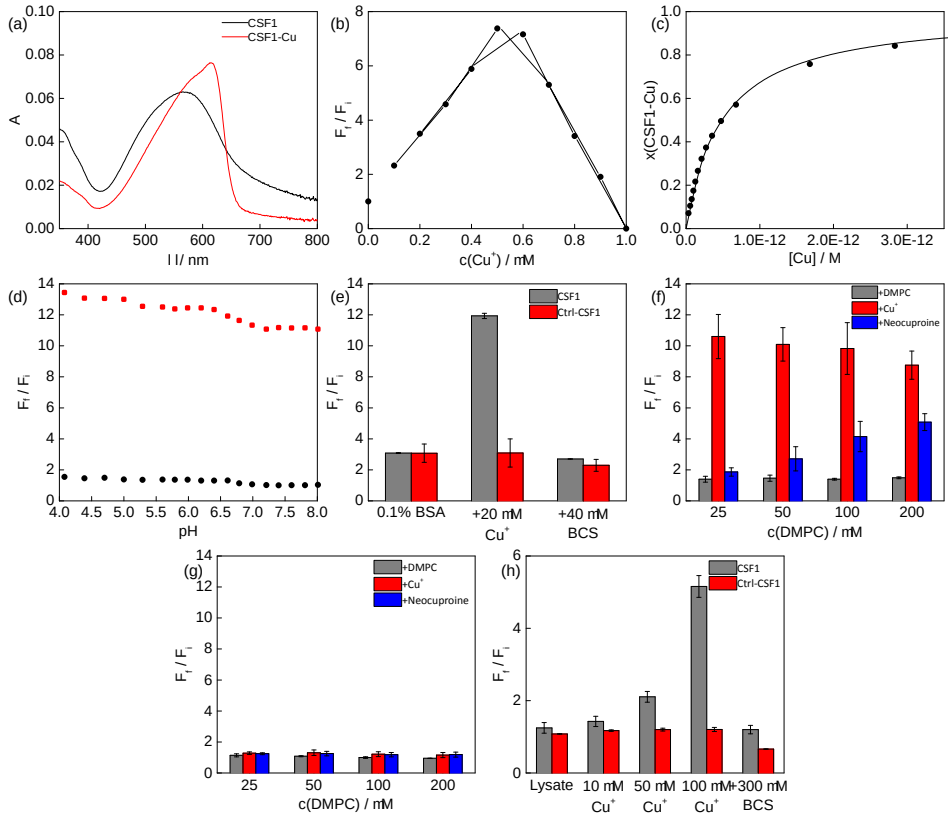


Figure S2. *In vitro* characterization of CSF1 and Ctrl-CSF1. For bar graphs, bars represent the final integrated fluorescence response (F_f) over the initial integrated emission (F_i) of CSF1 or Ctrl-CSF1 over 626–700 nm; values are shown as average ± s.d. (n=3). (a) UV-visible spectral change of 2 μM CSF1 (black) upon addition of 2 μM Cu^+ (red). (b) Job's plot of CSF1 and Cu^+. The total concentrations of CSF1 and Cu^+ were kept at 1 μM. (c) Fluorescence response of 1 μM CSF1 to thiourea-buffered Cu^+ solutions for K_d measurement. The observed K_d value is 4.9×10^{-13} M. Solid line represents the calculated curve. (d) Fluorescence intensity of 1 μM CSF1 (black) and 1 μM CSF1-Cu (red) across a range of pH values. (e) Fluorescence response of 1 μM CSF1 (grey) or Ctrl-CSF1 (red) to 0.1% BSA and subsequent addition of 20 μM Cu^+ and 40 μM BCS. (f) Fluorescence response of 1 μM CSF1 in the presence of DMPC (grey), with subsequent addition of 1 μM Cu^+ to the solution (red), and a final addition of 10 μM neocuproine (blue). (g) Fluorescence response of 1 μM Ctrl-CSF1 in the presence of DMPC (grey), with subsequent addition of 1 μM Cu^+ to the solution (red), and a final addition of 10 μM neocuproine (blue). (h) Fluorescence response of 1 μM CSF1 (grey) and Ctrl-CSF1 (red) to HEK293T lysates (1 mg protein/mL) and subsequent addition of 10, 50 and 100 μM Cu^+, followed by addition of 300 μM BCS.

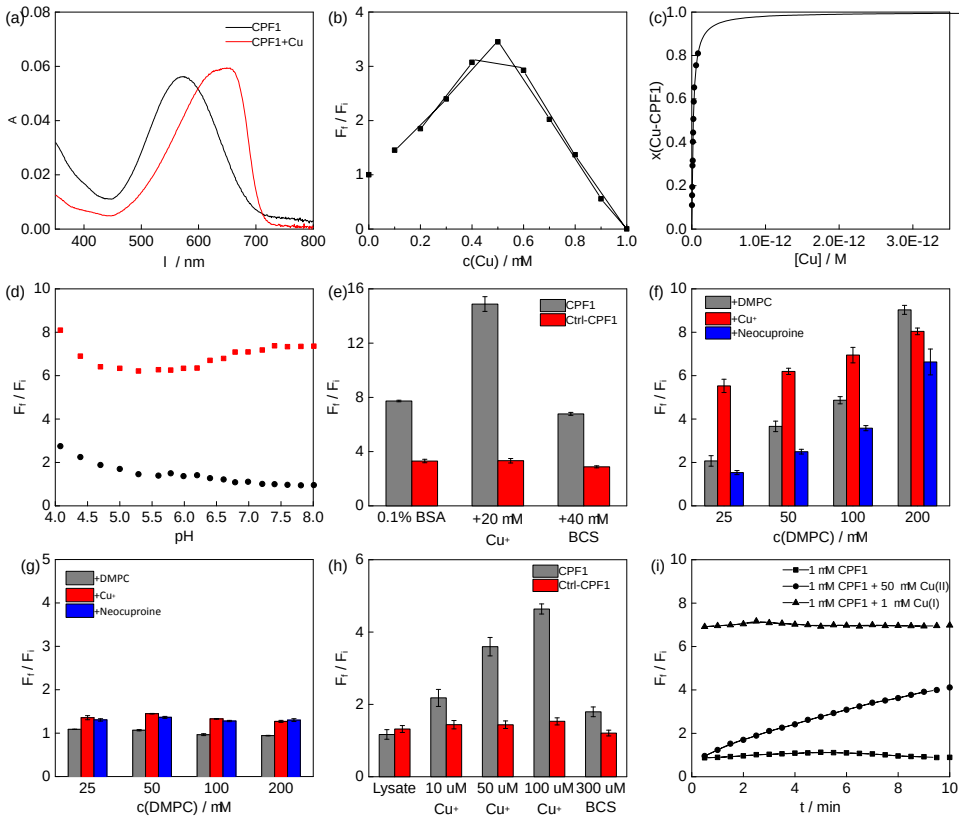


Figure S3. *In vitro* characterization of CPF1 and Ctrl-CPF1. For bar graphs, bars represent the final integrated fluorescence response (F_f) over the initial integrated emission (F_i) of CPF1 or Ctrl-CPF1 over 660–700 nm; values are shown as average ± s.d. (n=3). (a) UV-visible spectral change of 3 μM CPF1 (black) upon addition of 3 μM Cu^+ (red). (b) Job's plot of CPF1 and Cu^+. The total concentrations of CPF1 and Cu^+ were kept at 1 μM. (c) Fluorescence response of 1 μM CPF1 to thiourea-buffered Cu^+ solutions for K_d measurement. The observed K_d value is 0.20×10^{-13} M. Solid line represents the calculated curve. (d) Fluorescence intensity of 1 μM CPF1 (black) and 1 μM CPF1-Cu (red) across a range of pH values. (e) Fluorescence response of 1 μM CPF1 (grey) or Ctrl-CPF1 (red) to 0.1% BSA and subsequent addition of 20 μM Cu^+ and 40 μM BCS. (f) Fluorescence response of 1 μM CPF1 in the presence of DMPC (grey), with subsequent addition of 1 μM Cu^+ to the solution (red), and a final addition of 10 μM neocuproine (blue). (g) Fluorescence response of 1 μM Ctrl-CPF1 in the presence of DMPC (grey), with subsequent addition of 1 μM Cu^+ to the solution (red), and a final addition of 10 μM neocuproine (blue). (h) Fluorescence response of 1 μM CPF1 (grey) and Ctrl-CPF1 (red) to HEK 293T lysates (1 mg protein/mL) and subsequent addition of 10, 50 and 100 μM Cu^+, followed by addition of 300 μM BCS. (i) Fluorescence emission of 1 μM CPF1 (square) over time, and its response to 50 μM $CuSO_4$ (circle) or 1 μM $[Cu(CH_3CN)_4]PF_6$ (triangle). The slow turn-on response to Cu(II) compared to the prompt and stable turn-on by Cu(I) suggests that Cu(II) may be slowly reduced to Cu(I) by interaction with the probe receptor, resulting in an attenuated turn-on response.

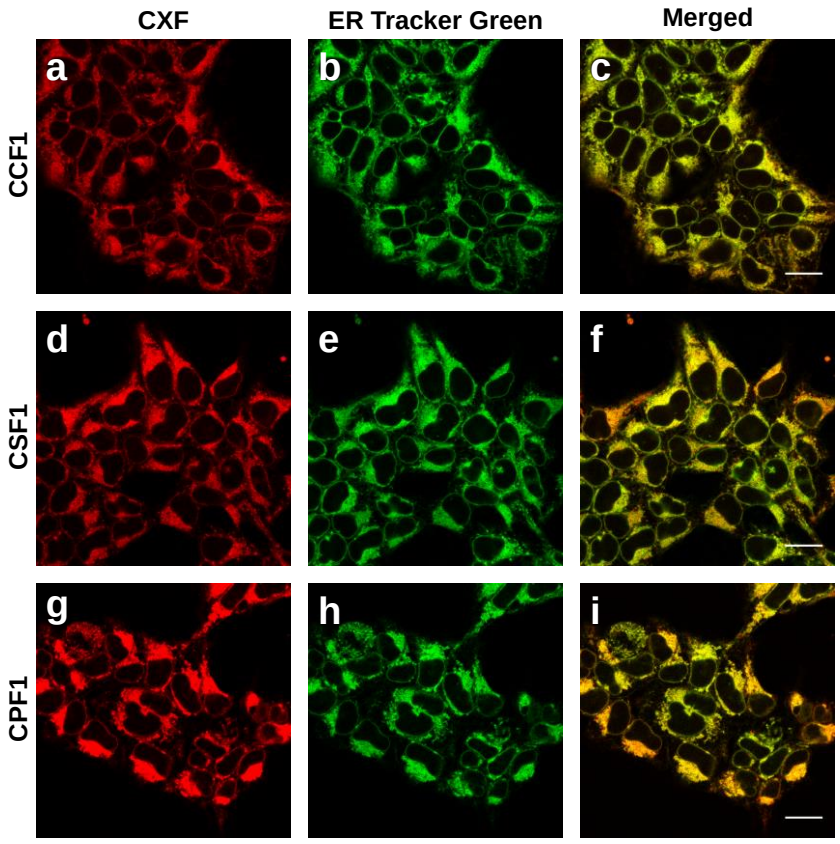


Figure S4. Colocalization of CCF1, CSF1 and CPF1 with ER-Tracker Green: (a) CCF1, (b) ER-Tracker Green, (c) merge of (a) and (b). (d) CSF1, (e) ER-Tracker Green, (f) Merge of (d) and (e). (g) CPF1, (h) ER-Tracker Green, (i) merge of (g) and (h). Scale-bars: 20 μm. Pearson's coefficients of pixel intensity spatial correlation between ER-Tracker Green and CCF1, CSF1 or CPF1 are 0.87 ± 0.03, 0.82 ± 0.04 and 0.85 ± 0.04, respectively, averaged across 3 separate fields of cells using Fiji's Coloc 2 plugin for ImageJ; error represents the standard deviation between different fields of cells.

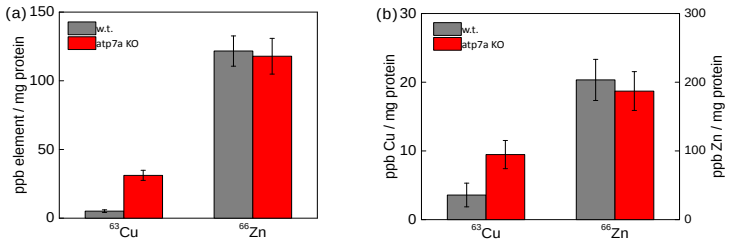


Figure S5. Detection of total copper and zinc pools in (a) cytoplasmic extract and (b) nuclear extract of Atp7a$^{-/-}$ MEFs and its genetically matched controls. Values are shown as mean ± sem (n=3).

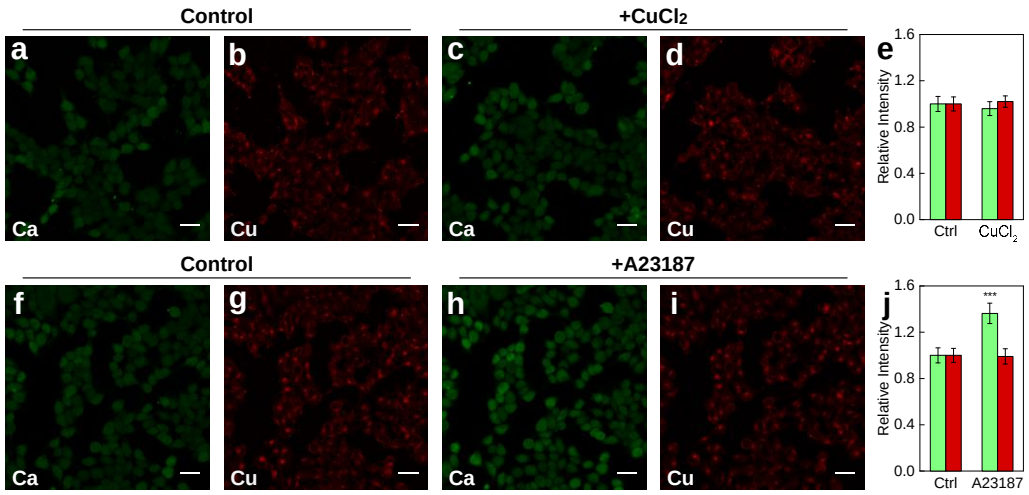
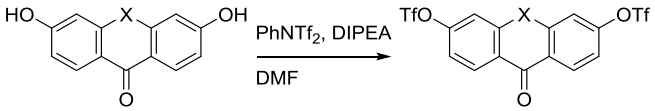


Figure S6. Dual-channel imaging in HEK 293T cells with Calcium Green-1 in the green channel (a, c, f, h) and Ctrl-CPF1 in the red channel (b, d, g, i). Control cells (a, b) and cells treated with 100 μM $CuCl_2$ for 12 h (c, d) were incubated with both dyes in HBSS and imaged; quantification is shown in (e). Cells incubated with both dyes in HBSS prior to (f, g) and after (h, i) treatment of 1 μM calcium ionophore A23187 were imaged; quantification is shown in (j). Green bars represent the Calcium Green-1 channel and red bars are Ctrl-CPF1 channel. Scale-bars: 40 μm. Data were normalized to controls cells and shown as average ± s.d. (n = 4). ***P $\leqslant$ 0.001; two-tailed Student's t-test..

Syntheses of CCF1/Ctrl-CCF1, CSF1/Ctrl-CSF1 and CPF1/Ctrl-CPF1

Synthetic materials and methods

All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry N_2. THF used for anhydrous reactions was dried and stored over 4 Å molecular sieves. 3,6-dihydroxy-10,10-dimethylanthracen-9(10*H*)-one (**S1a**),[2] 3,7-dihydroxy-5,5-dimethyldibenzo[b,e]silin-10(5*H*)-one (**S1b**)[3] and 3,7-dihydroxy-5-phenyl-10*H*-acridophosphin-10-one 5-oxide (**S1c**)[4], *N*-(4-Bromo-3-(trifluoromethyl)benzyl)-*N*,*N*-bis(2-((2-(ethylthio)ethyl)thio)ethyl)amine (**S5**) and *N*-(4-Bromo-3-(trifluoromethyl)benzyl)-*N*,*N*-dioctylamine (**S6**) were synthesized according to literature procedure.[5] All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were collected in $CDCl_3$ or CD_3OD (Cambridge Isotope Laboratories, Cambridge, MA) at 25 °C on AVB-400, AVQ-400 or DRX-500 spectrometers at the College of Chemistry NMR Facility at the University of California, Berkeley. All chemical shifts are reported in the standard δ notation of ppm relative to residual solvent peak ($CDCl_3$ δH=7.26, δC=77.20; CD_3OD δH=3.31, δC=49.00). Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets. Low-resolution electrospray mass spectral analyses were carried out using a LC-MS (Advion expression-L Compact Mass Spectrometer). Low resolution and high resolution electron ionization mass spectral analyses were carried out at the College of Chemistry Mass Spectrometry Facility at the University of California, Berkeley. High resolution mass spectral analyses (ESI-MS) were carried out at LBNL Catalysis Facility at the Lawrence Berkeley National Laboratory (Berkeley Lab) using a UHPLC-TOF (PerkinElmer AxION® 2 TOF MS).



S1a: X = $C(CH_3)_2$
S1b: X = $Si(CH_3)_2$
S1c: X = P=O(Ph)

S2a: X = $C(CH_3)_2$
S2b: X = $Si(CH_3)_2$
S2c: X = P=O(Ph)

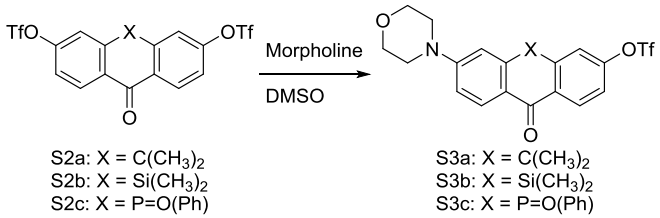
General procedure: A solution of **S1**, $PhNTf_2$ and DIPEA in anhydrous DMF was stirred at room temperature overnight. The reaction was diluted with H_2O, transferred into a separatory funnel and extracted with EtOAc. The organic layer was washed with H_2O (×4), brine, then dried (Na_2SO_4) and concentrated under reduced pressure. The resultant residue was purified using flash chromatography (silica gel) to give the product.

9,9-Dimethyl-10-oxo-9,10-dihydroanthracene-2,7-diyl bis(trifluoromethanesulfonate) (S2a). Following general procedure, **S1a** (4.0 g, 15.90 mmol), $PhNTf_2$ (17.04 g, 47.69 mmol) and DIPEA (16.61 mL, 95.38 mmol) was reacted in DMF (60 mL) to provide **S2a** (5.69 g, 69%) as an off-white

solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 2.3 Hz, 2H), 7.38 (dd, *J* = 8.8, 2.3 Hz, 2H), 1.78 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.91, 153.37, 152.68, 130.95, 129.43, 120.58, 120.01, 117.32, 38.78, 33.00. HRMS (EI^+) m/z calcd 517.9929, found 517.9922 for $C_{18}H_{12}F_6O_7S_2^+$ (M^+).

5,5-Dimethyl-10-oxo-5,10-dihydrodibenzo[b,e]siline-3,7-diyl bis(trifluoromethanesulfonate) (S2b). Following general procedure, **S1b** (553 mg, 2.03 mmol), $PhNTf_2$ (2.18g, 6.10 mmol) and DIPEA (2.12 mL, 12.2 mol) was reacted in DMF (6 mL) to provide **S2b** (1.09g, 100%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, 2H, J=8.8 Hz), 7.54 (d, 2H, J=2.5 Hz), 7.48 (dd, 2H, J=2.6, 8.8 Hz), 0.58 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): 185.06, 152.28, 142.15, 140.06, 133.24, 130.15, 125.58, 123.43, -1.61. HRMS (EI^+): m/z calcd 533.9698, found 533.9697 for $C_{17}H_{12}F_6O_7S_2Si^+$ (M^+).

5-Oxido-10-oxo-5-phenyl-10H-acridophosphine-3,7-diyl bis(trifluoromethanesulfonate) (S2c). Following general procedure, **S1c** (190 mg, 0.56 mmol), $PhNTf_2$ (605.5 mg, 1.68 mmol) and DIPEA (0.57 mL, 3.36 mmol) gave, was reacted in DMF (2.5 mL) to provide **S2c** (269 mg, 80%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 8.8, 5.1 Hz, 2H), 7.92 (dd, *J* = 13.1, 2.6 Hz, 2H), 7.68 (dd, *J* = 8.8, 2.5 Hz, 2H), 7.58 – 7.42 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 180.1, 180.0, 153.0. 152.9, 136.8, 135.9, 134.9, 134.8, 133.1, 132.8, 132.7, 131.3, 130.7, 130.6, 130.2, 129.5, 129.4, 126.1, 123.9, 120.2, 117.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 2.4. LRMS (EI^+): m/z calcd. 600, found 600 for $C_{21}H_{11}F_6O_8PS_2$ (M^+).



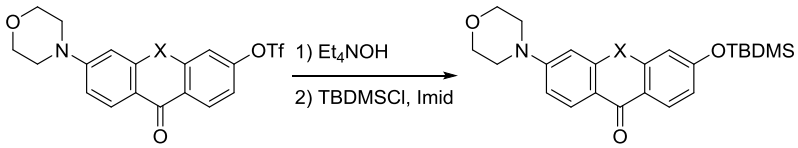
General procedure: Morpholine was added to a solution of **S2** in DMSO. The solution was stirred overnight at 90 °C. After cooling to room temperature, the reaction was diluted with H_2O and extracted with EtOAc. The organic layer was washed with H_2O (×4), brine, then dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified using flash chromatography (silica gel) to give unreacted starting material and the product.

9,9-Dimethyl-7-morpholino-10-oxo-9,10-dihydroanthracen-2-yl trifluoromethanesulfonate (S3a). Following general procedure, **S2a** (1.13 g, 2.18 mmol) and morpholine (188.1 µL, 2.18 mmol) was reacted in DMSO (10.9 mL) to provide **S3a** (0.49 g, 49%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, *J* = 8.7 Hz, 1H), 8.26 (d, *J* = 8.8 Hz, 1H), 7.53 (d, *J* = 2.4 Hz, 1H), 7.31 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.96 (d, *J* = 7.8 Hz, 2H), 3.89 (t, *J* = 4.7 Hz, 4H), 3.39 (t, *J* = 4.8 Hz, 4H), 1.72 (s, 6H). ^{13}C NMR

(101 MHz, $CDCl_3$) δ 180.38, 154.67, 152.81, 152.33, 151.76, 130.11, 129.91, 129.60, 120.90, 120.20, 119.45, 117.00, 113.49, 110.01, 66.36, 47.29, 38.22, 32.91. LRMS (ESI^+): m/z cald 456.1, found 456.0 for $C_{21}H_{21}F_3NO_5S^+$ ($M+H^+$).

5,5-Dimethyl-7-morpholino-10-oxo-5,10-dihydrodibenzo[b,e]siline-3-yl trifluoromethanesulfonate (S3b). Following general procedure, **S2b** (570 mg, 1.07 mmol) and morpholine (92.2 µL, 1.07 mmol) was reacted in DMSO (2 mL) to provide **S3b** (227 mg, 45%), as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.54 (d, 1H, J=8.8 Hz), 8.37 (d, 1H, J=9.0 Hz), 7.49 (d, 1H, J=2.6 Hz), 7.42 (dd, 1H, J=2.6, 8.8 Hz), 7.05 (dd, 1H, J=2.7, 9.0 Hz), 7.00 (d, 1H, J=2.6 Hz), 3.89 (t, 4H, J=5.0 Hz), 3.38 (t, 4H, J=5.0 Hz), 0.51 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 184.9, 153.1, 151.8, 142.6, 141.0, 140.4, 132.7, 132.4, 131.1, 125.2, 122.8, 120.5, 116.9, 116.0, 66.8, 47.4, -1.31. HRMS (ESI^+): m/z calcd 472.0856, found 472.0849 for $C_{20}H_{21}F_3NO_5SSi^+$ ($M+H^+$).

7-Morpholino-5-oxido-10-oxo-5-phenyl-10H-acridophosphine-3-yl trifluoromethanesulfonate (S3c). Following general procedure, **S2c** (269 mg, 0.45 mmol) and morpholine (39.2 µL, 0.45 mmol) was reacted in DMSO (0.5 mL) to provide **S3c** (147 mg, 61%) as a yellow, thick liquid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.52 (dd, *J* = 8.8, 5.1 Hz, 1H), 8.35 (dd, *J* = 9.1, 5.9 Hz, 1H), 7.88 (dd, *J* = 12.7, 2.6 Hz, 1H), 7.62 – 7.36 (m, 6H), 7.32 (dd, *J* = 15.1, 2.7 Hz, 1H), 7.12 (dd, *J* = 9.1, 2.7 Hz, 1H), 3.82 (t, *J* = 4.9 Hz, 4H), 3.45 – 3.33 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$-*d*): δ 179.6, 179.5, 153.9, 153.8, 152.3, 152.2, 137.2, 136.3, 135.8, 135.0, 134.0, 133.2, 132.4, 132.3, 132.1, 132.0, 130.6, 130.5, 129.1, 129.0, 125.4, 125.3, 125.2, 123.3, 123.2, 116.9, 114.1, 114.0, 66.3, 46.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 3.9. LRMS (ESI^+): m/z calcd 538.1, found 538.3 for $C_{24}H_{20}F_3NO_6PS^+$ ($M+H^+$).



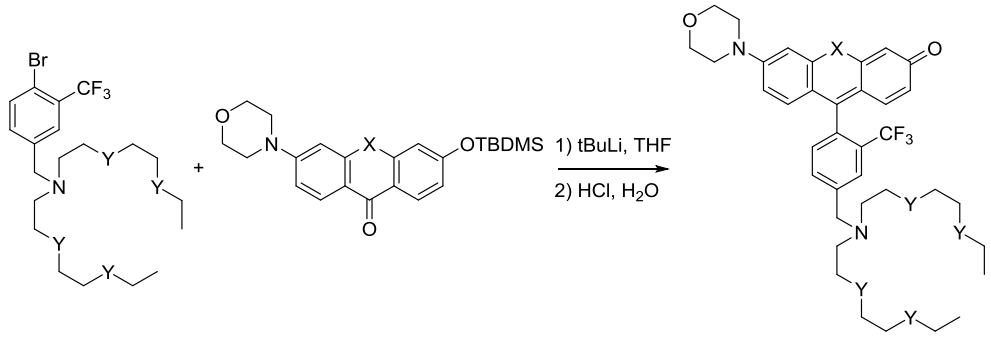
General procedure: A solution of **S3** in dioxane was treated with a solution of Et_4NOH in MeOH. The resultant solution was stirred at room temperature for 2 h. After the reaction was complete, the volatiles were removed under reduced pressure, and the residue was dissolved in CH_2Cl_2. The solution was cooled to 0 °C followed by treatment with imidazole and tertbutyldimethylsilyl chloride. After stirring for 4 h at room temperature, the reaction was diluted with CH_2Cl_2 and washed with H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was purified using flash chromatography (silica gel) to give the product.

3-((*Tert*-Butyldimethylsilyl)oxy)-10,10-dimethyl-6-morpholinoanthracen-9(10*H*)-one (S4a).

Following general procedure, **S3a** (230 mg, 0.57 mmol) was reacted with Et_4NOH (1.0 mL, 1.5 M in methanol, 1.5 mmol) in dioxane (5 mL) and imidazole (0.10 g, 1.53 mmol), *tert*-butyldimethylsilyl chloride (0.15 g, 1.02 mmol) in CH_2Cl_2 (12.8 mL) to give **S4a** (200 mg, 92%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 8.7, 3.2 Hz, 2H), 7.02 (dd, *J* = 12.3, 2.4 Hz, 2H), 6.91 (ddd, *J* = 29.7, 8.8, 2.4 Hz, 2H), 3.89 (t, *J* = 4.9 Hz, 4H), 3.36 (t, *J* = 4.9 Hz, 4H), 1.68 (s, 6H), 1.01 (s, 9H), 0.26 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.51, 159.91, 154.22, 152.57, 152.13, 129.44, 129.27, 124.43, 122.10, 118.95, 117.34, 113.42, 110.60, 66.58, 47.78, 37.92, 33.18, 25.61, 18.25, -4.33. LRMS (ESI^+): m/z cald 438.2, found 438.1 for $C_{26}H_{36}NO_3Si^+$ ($M+H^+$).

3-((*tert*-Butyldimethylsilyl)oxy)-5,5-dimethyl-7-morpholinodibenzo[b,e]silin-10(5H)-one (S4b). Following general procedure, **S3b** (149 mg, 0.32 mmol) was reacted with Et_4NOH (0.42 mL, 1.5 M in methanol, 0.63 mmol) in dioxane (10 mL) and imidazole (215 mg, 3.16 mmol), *tert*-butyldimethylsilyl chloride (143 mg, 0.95 mmol) in CH_2Cl_2 (15 mL) to give **S4b** (100 mg, 70%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (d, *J* = 8.4 Hz, 2H), 7.05−6.98 (m, 4H), 3.89 (t, *J* = 4.8 Hz, 4H), 3.36 (t, *J* = 4.8 Hz, 4H), 1.02 (s, 9H), 0.47 (s, 6H), 0.27 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 185.75, 158.75, 152.64, 141.20, 140.78, 143.80, 132.24, 131.93, 123.82, 121.85, 117.19, 115.91, 66.78, 47.74, 25.82, 18.46, -1.18, -4.13. HRMS (ESI^+): m/z calcd 454.2228, found 454.2219 for $C_{25}H_{36}NO_3Si_2^+$ ($M+H^+$).

3-((*tert*-Butyldimethylsilyl)oxy)-7-morpholino-5-phenyl-10H-acridophosphin-10-one 5-oxide (S4c). Following general procedure, **S3c** (110 mg, 0.21 mmol) was reacted with Et_4NOH (0.28 mL, 1.5 M) in dioxane (6 mL) and imidazole (142 mg, 2.1 mmol), *tert*-butyldimethylsilyl chloride (142 mg, 2.1 mmol) in CH_2Cl_2 (15 mL) to give **S4c** (85 mg, 78%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 – 8.11 (m, 2H), 7.67 – 7.48 (m, 2H), 7.44 – 7.29 (m, 5H), 7.18 – 6.86 (m, 2H), 3.80 (t, *J* = 4.9 Hz, 4H), 3.41 – 3.29 (m, 4H), 0.93 (s, 9H), 0.18 (d, *J* = 5.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$-*d*): δ 180.4, 169.0. 167.3, 164.8, 160.1, 153.5, 139.2, 135.3, 131.7, 131.5, 131.3, 130.5, 130.4, 130.0, 129.6, 128.7, 128.6, 126.2, 124.0, 121.6, 121.5, 116.8, 114.2, 114.1, 66.3, 46.9, 25.5, 18.1, -4.39. ^{31}P NMR (162 MHz, $CDCl_3$) δ 4.8. LRMS (ESI^+): m/z calcd 520.2, found 520.5 for $C_{29}H_{35}NO_4PSi$ ($M+H^+$).



S5: Y = S
S6: Y = CH_2

S4a: X = $C(CH_3)_2$
S4b: X = $Si(CH_3)_2$
S4c: X = P=O(Ph)

CCF1: X = $C(CH_3)_2$, Y=S
CtrlCCF1: X = $C(CH_3)_2$, Y=CH_2
CSF1: X = $Si(CH_3)_2$, Y=S
CtrlCSF1: X = $Si(CH_3)_2$, Y=CH_2
CPF1: X = P=O(Ph), Y=S
CtrlCPF1: X = P=O(Ph), Y=CH_2

General procedure: A flame-dried flask charged with **S5** or **S6** in dry THF (1 mL) was cooled to -78 °C. A solution of *tert*-butyllithium in pentane was added drop-wise under nitrogen. After stirring at the same temperature for 10 to 20 min, a solution of **S4** in dry THF (2 to 4 mL) was added. The resultant solution was warmed to room temperature and stirred for 60 min. Aqueous HCl (20 mL, 1 M) was added to the reaction and stirred for an additional 60 min. The reaction was neutralized with $NaHCO_3$ and extracted with EtOAc (×3). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified via flash chromatography to give the product.

CCF1. Following general procedure, **S5** (215 mg, 0.39 mmol) was reacted with *tert*-butyllithium (0.46 mL, 1.7 M in pentane, 0.78 mmol) and **S4a** (85 mg, 0.20 mmol) to give **CCF1** (81 mg, 53%) as a dark magenta solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 1.5 Hz, 1H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 8.3 Hz, 1H), 7.09 (d, *J* = 1.7 Hz, 1H), 6.79 (d, *J* = 9.8 Hz, 1H), 6.77 (d, *J* = 1.8 Hz, 1H), 6.65 (s, 2H), 6.30 (dd, *J* = 9.7, 1.9 Hz, 1H), 3.92 – 3.78 (m, 6H), 3.40 – 3.29 (m, 4H), 2.86 – 2.83 (m, 4H), 2.76 – 2.72 (m, 12H), 2.56 (q, *J* = 7.4 Hz, 4H), 1.73 (s, 3H), 1.57 (s, 3H), 1.25 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.25, 156.13, 152.97, 150.01, 149.37, 138.08, 132.55, 131.80, 131.66, 129.72, 126.64, 124.61, 123.28, 122.86, 112.41, 111.32, 77.52, 77.20, 76.88, 66.68, 58.03, 54.06, 47.57, 40.29, 35.99, 32.72, 31.96, 30.61, 30.34, 26.28, 14.98. HRMS (ESI^+) m/z calcd 777.2858, found 777.2869 for $C_{40}H_{52}F_3N_2O_2S_4^+$ ($M+H^+$).

Ctrl-CCF1. Following general procedure, **S6** (100 mg, 0.21 mmol) was reacted with *tert*-butyllithium (0.25 mL, 1.7 M in pentane, 0.42 mmol) and **S4a** (46 mg, 0.11 mmol) to give **Ctrl-CCF1** (44 mg, 60%) as a dark magenta solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.64 (s, 1H), 7.21 (d, *J* = 7.7 Hz, 1H), 7.09 (d, *J* = 2.2 Hz, 1H), 6.79 (d, *J* = 9.7 Hz, 1H), 6.74 (d, *J* = 1.8 Hz, 1H), 6.66 (d, *J* = 8.9 Hz, 1H), 6.60 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.27 (dd, *J* = 9.8, 1.8 Hz, 1H), 3.92 – 3.77 (m, 4H), 3.69 (s, 2H), 3.38 – 3.25 (m, 4H), 1.73 (s, 3H), 1.57 (s, 3H), 1.37 – 1.15 (m, 33H), 0.86 (dd, *J* = 8.8, 4.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.50, 156.13, 152.93, 149.90, 138.13, 132.47, 131.40, 126.74, 124.73, 123.38, 123.06, 112.34, 111.40, 77.55, 77.23, 76.92, 66.73, 60.59, 58.35, 54.33, 47.63, 40.28, 36.01, 32.05, 30.61, 29.90, 29.71, 29.51, 27.62, 27.30, 22.86, 14.39, 14.30. HRMS (ESI^+) m/z calcd 705.4601, found 705.4604 for $C_{44}H_{60}F_3N_2O_2^+$ ($M+H^+$).

CSF1. Following general procedure, **S5** (55 mg, 0.099 mmol) was reacted with *tert*-butyllithium (0.12 mL, 1.7 M in pentane, 0.20 mmol) and **S4b** (30 mg, 0.066 mmol) to give **CSF1** (23 mg, 29%) as a dark purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.70 (s, 1H), 7.23 (d, *J* = 7.7 Hz, 1H), 7.09 (d, *J* = 2.6 Hz, 1H), 6.83 (d, *J* = 2.1 Hz, 1H), 6.75 (d, *J* = 10.1 Hz, 1H), 6.67 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.62 (d, *J* = 9.1 Hz, 1H), 6.21 (dd, *J* = 10.1, 2.1 Hz, 1H), 3.91 – 3.75 (m, 6H), 3.39 – 3.24 (m, 4H), 2.84 (s, 4H), 2.73 (s, 12H), 2.56 (q, *J* = 7.4 Hz, 4H), 1.25 (t, *J* = 7.4 Hz, 1H), 0.52 (s, 3H), 0.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.38, 150.78, 146.95, 141.61, 140.15, 136.70, 135.48, 131.81, 131.67, 128.51, 127.20, 119.44, 114.47, 77.40, 66.67, 58.02, 54.07, 47.18, 32.74, 31.98, 30.35, 26.29, 14.98, -0.26, -2.04. HRMS (ESI^+) m/z calcd 793.2627, found 793.2624 for $C_{39}H_{52}F_3N_2O_2S_4Si^+$ ($M+H^+$).

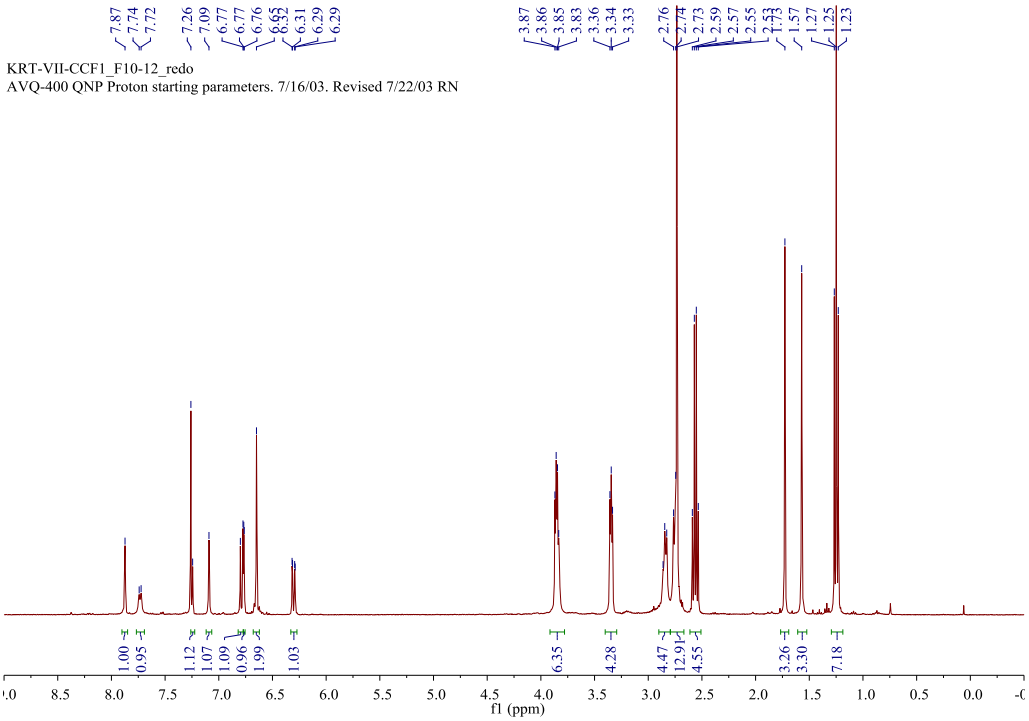
Ctrl-CSF1. Following general procedure, **S6** (47 mg, 0.099 mmol) was reacted with *tert*-butyllithium (0.12 mL, 1.7 M in pentane, 0.20 mmol) and **S4b** (30 mg, 0.066 mmol) to give **Ctrl-CSF1** (18 mg, 25%) as a dark purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.62 (s, 1H), 7.20 (s, 1H), 7.09 (s, 1H), 6.83 (d, *J* = 2.1 Hz, 1H), 6.76 (d, *J* = 8.3 Hz, 1H), 6.62 (s, 2H), 6.20 (dd, *J* = 10.1, 2.1 Hz, 1H), 3.88 – 3.81 (m, 4H), 3.68 (s, 2H), 3.36 – 3.28 (m, 4H), 2.48 (s, 3H), 1.37 – 1.12 (m, 24H), 0.86 (t, J = 6.2 Hz, 6H), 0.49 (d, J = 16.9 Hz, 3H), 0.42 (s, 3H). 13C NMR (101 MHz, CDCl3) δ 184.46, 150.78, 147.04, 141.74, 140.19, 136.66, 135.48, 131.75, 128.52, 127.15, 119.47, 114.35, 77.40, 66.67 , 51.01, 47.18, 32.00, 29.64, 29.46, 27.53, 22.83, 14.36, -0.27, -2.05. HRMS (ESI^+) m/z calcd 721.4371, found 721.4358 for $C_{43}H_{60}F_3N_2O_2Si^+$ ($M+H^+$).

CPF1. Following general procedure, **S5** (127 mg, 0.23 mmol) was reacted with *tert*-butyllithium (0.30 mL 1.7 M in pentane, 0.46 mmol) and **S4c** (40 mg, 0.077 mmol) to give **CPF1** (57 mg, 38 %) as a dark blue solid. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ 8.06 (s, 1H), 7.86 (d, *J* = 7.7 Hz, 1H), 7.73 – 7.40 (m, 6H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.11 (dd, *J* = 16.9, 2.0 Hz, 1H), 7.00 (dd, *J* = 9.1, 2.8 Hz, 1H), 6.89 (dd, *J* = 10.0, 6.8 Hz, 1H), 6.82 (dd, *J* = 9.2, 6.3 Hz, 1H), 6.29 (dd, *J* = 10.1, 2.1 Hz, 1H), 3.86 (s, 2H), 3.76 (t, *J* = 4.8 Hz, 4H), 3.45 (t, *J* = 4.9 Hz, 4H), 2.84 – 2.73 (m, 8H), 2.71 (s, 8H), 2.54 (q, *J* = 7.4 Hz, 4H), 1.20 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (125 MHz, CD_3OD): δ 183.9, 183.8, 152.4, 152.3, 151.8, 142.5, 140.6, 140.2, 139.5, 136.0, 133.9, 133.4, 132.7, 132.4, 131.7, 129.8, 129.7, 129.0, 128.9, 128.4, 128.2, 126.7, 125.6, 125.1, 123.8, 122.9, 122.6, 116.6, 115.9, 66.0, 57.3, 53.8, 46.3, 31.9, 31.4, 29.6, 25.3, 13.9. ^{31}P NMR (162 MHz, CD_3OD) δ 10.4. HRMS (ESI^+) m/z calcd 859.2467, found 859.2454 for $C_{43}H_{51}F_3N_2O_3PS_4^+$ ($M+H^+$).

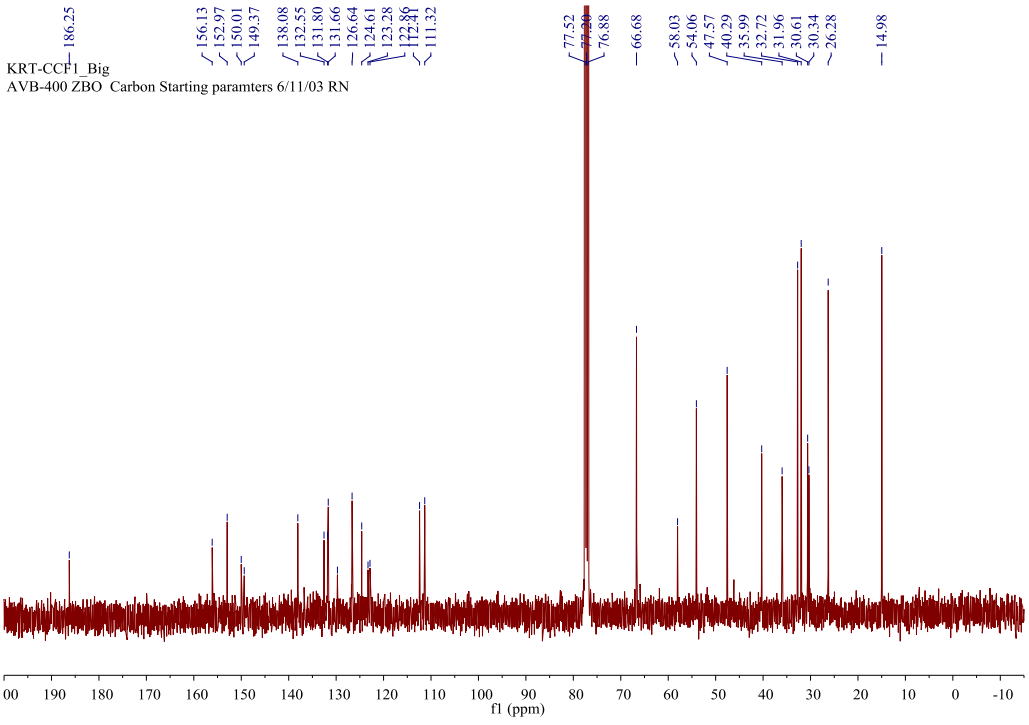
Ctrl-CPF1. Following general procedure, **S6** (70 mg, 0.14 mmol) was reacted with *tert*-butyllithium (0.18 mL 1.7 M in pentane, 0.29 mmol) and **S4c** (25 mg, 0.048 mmol) to give **CPF1** (14 mg, 37 %) as a dark blue solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (s, 1H), 7.78 (d, *J* = 7.1 Hz, 1H), 7.69 – 7.42 (m, 6H), 7.38 (d, *J* = 7.7 Hz, 1H), 7.11 (dd, *J* = 16.9, 2.1 Hz, 1H), 6.96 (dd, *J* = 9.3, 2.8 Hz, 1H), 6.83 (ddd, *J* = 18.9, 9.7, 6.5 Hz, 2H), 6.26 (dd, *J* = 10.0, 2.2 Hz, 1H), 3.80 (s, 2H), 3.75 (t, *J* = 4.9 Hz, 4H), 3.44 (t, *J* = 4.9 Hz, 4H), 2.55 (t, *J* = 7.2 Hz, 4H), 1.54 (dd, *J* = 10.4, 3.7 Hz, 4H), 1.33 – 1.16 (m, 20H), 0.94 – 0.75 (m, 6H).^{13}C NMR (101 MHz, CD_3OD) δ 183.9, 183.7, 152.4, 152.3, 151.6, 151.6, 142.3, 140.3, 140.3, 139.4, 136.0, 135.8, 134.0, 133.3, 132.7, 132.5, 132.2, 131.7, 131.6, 129.8, 129.7, 129.0, 128.8, 128.5, 128.2, 126.7, 125.6, 123.8, 122.6, 116.7, 115.8, 65.9, 57.6, 53.7, 46.3, 31.6, 29.2, 29.1, 27.1, 26.5, 22.3, 13.0. ^{31}P NMR (162 MHz, CD_3OD) δ 10.4. HRMS (ESI^+) m/z calcd 787.4210, found 787.4202 for $C_{47}H_{59}F_3N_2O_3P^+$ ($M+H^+$).

^{1}H and ^{13}C NMR spectra

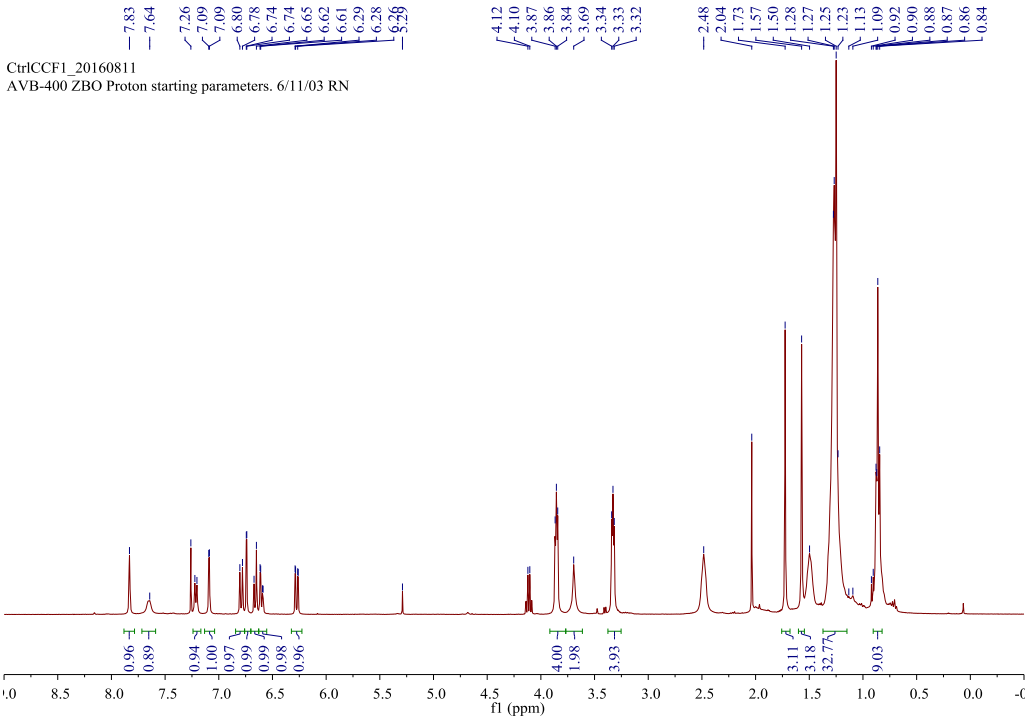
^{1}H NMR spectrum of CCF1 ($CDCl_3$, 400 MHz):



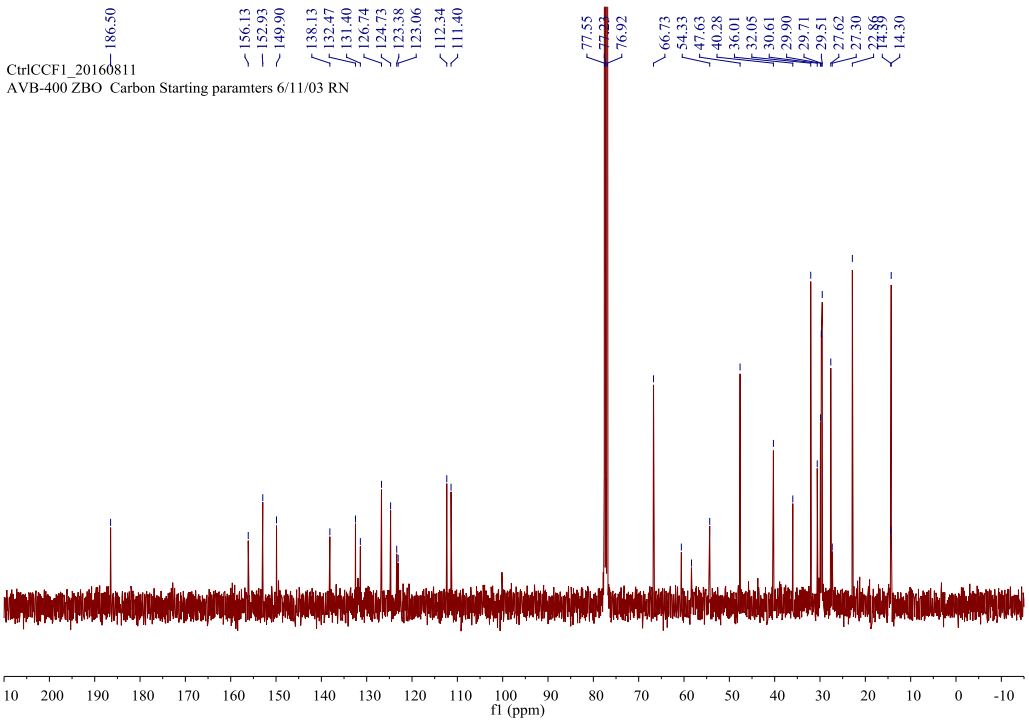
^{13}C NMR spectrum of CCF1 ($CDCl_3$, 101 MHz):



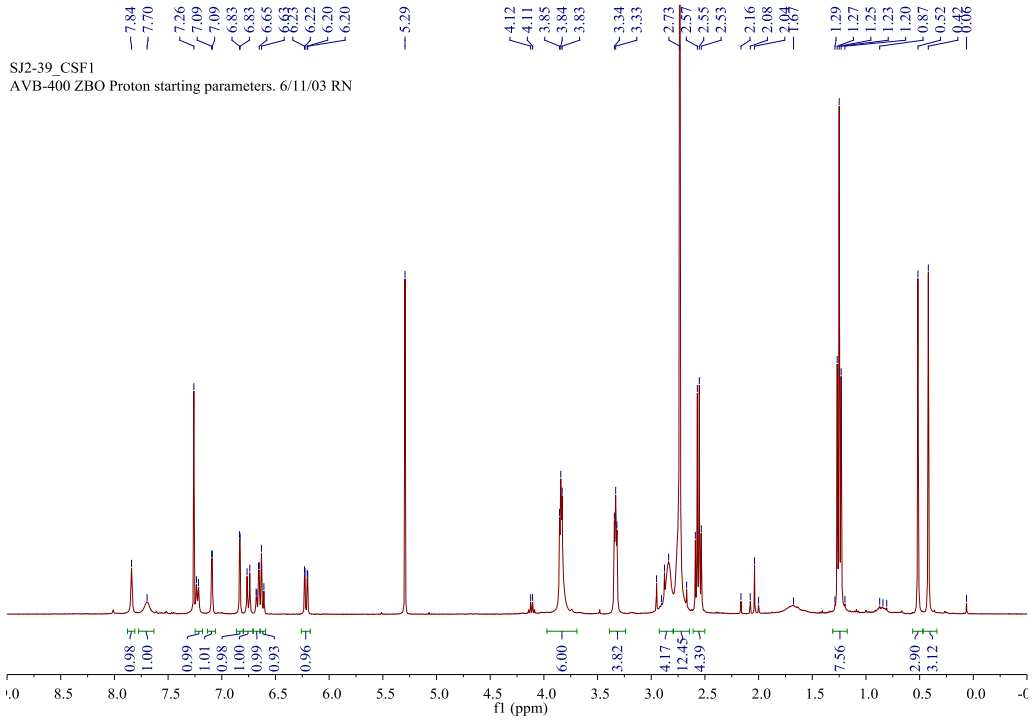
^{1}H NMR spectrum of Ctrl-CCF1 ($CDCl_3$, 400 MHz):



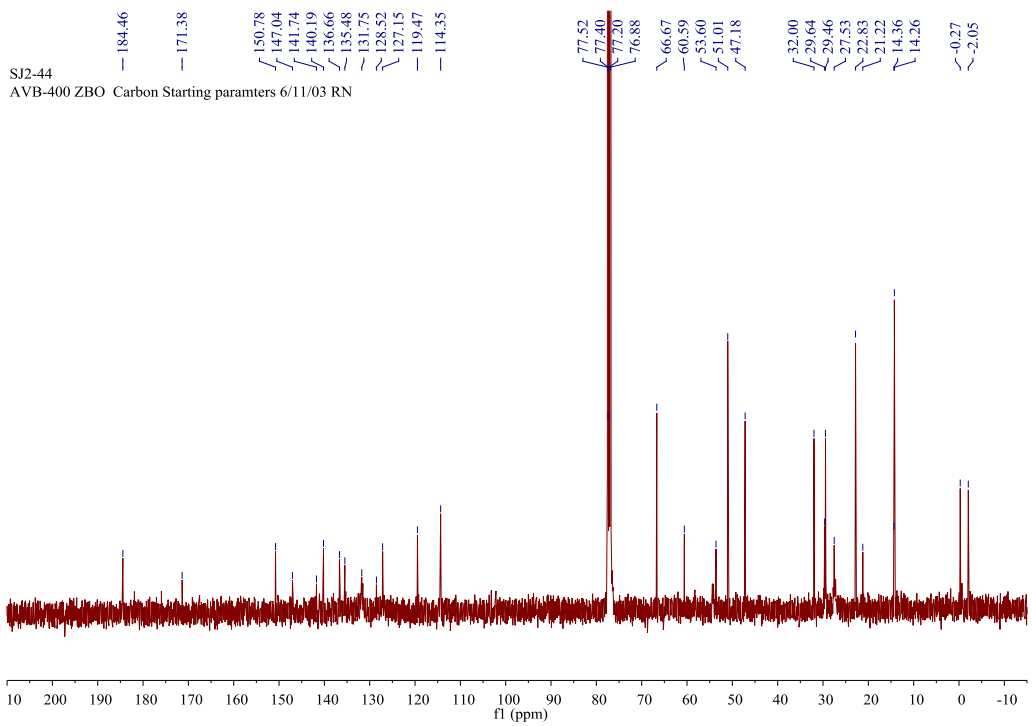
^{13}C NMR spectrum of Ctrl-CCF1 ($CDCl_3$, 101 MHz):



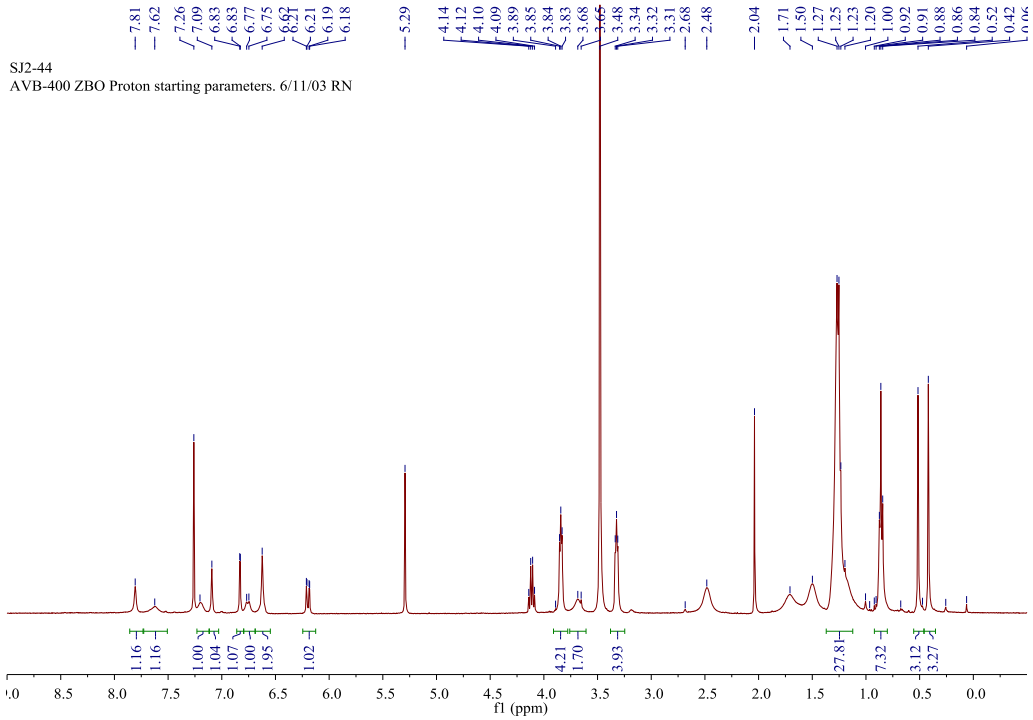
^{1}H NMR spectrum of CSF1 ($CDCl_3$, 400 MHz):



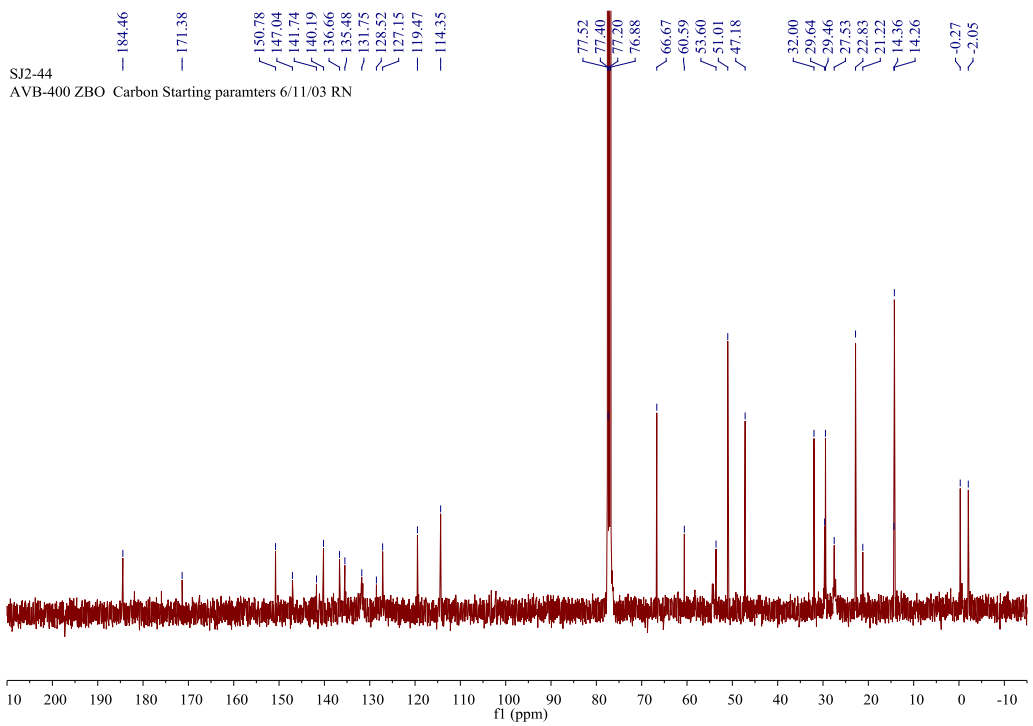
^{13}C NMR spectrum of CSF1 ($CDCl_3$, 101 MHz):



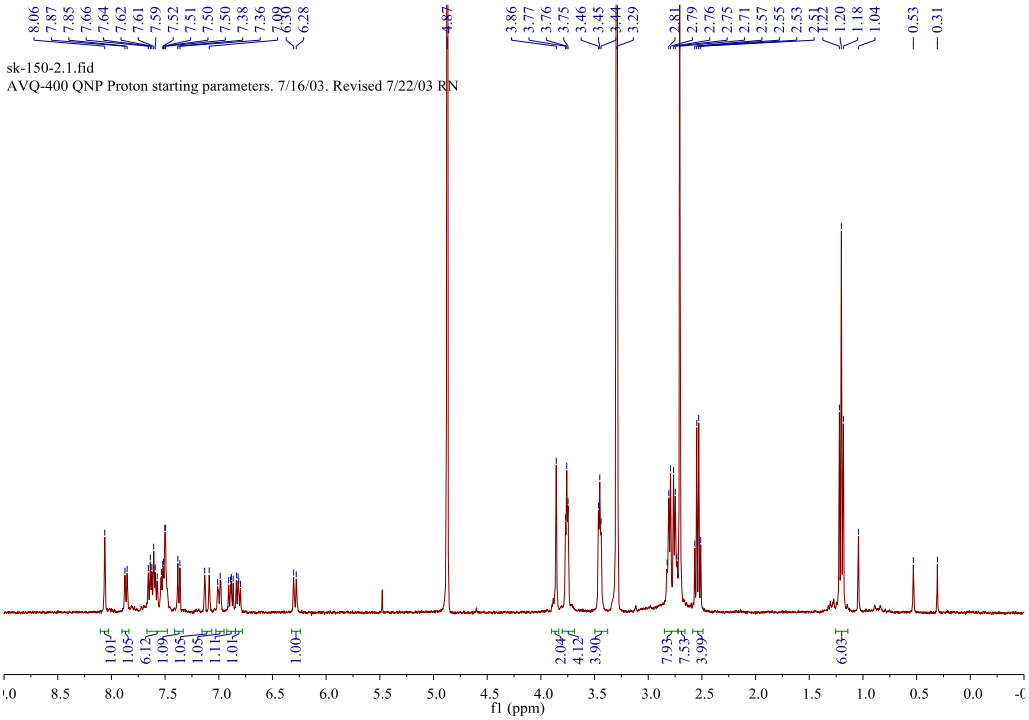
^{1}H NMR spectrum of Ctrl-CSF1 ($CDCl_3$, 400 MHz):



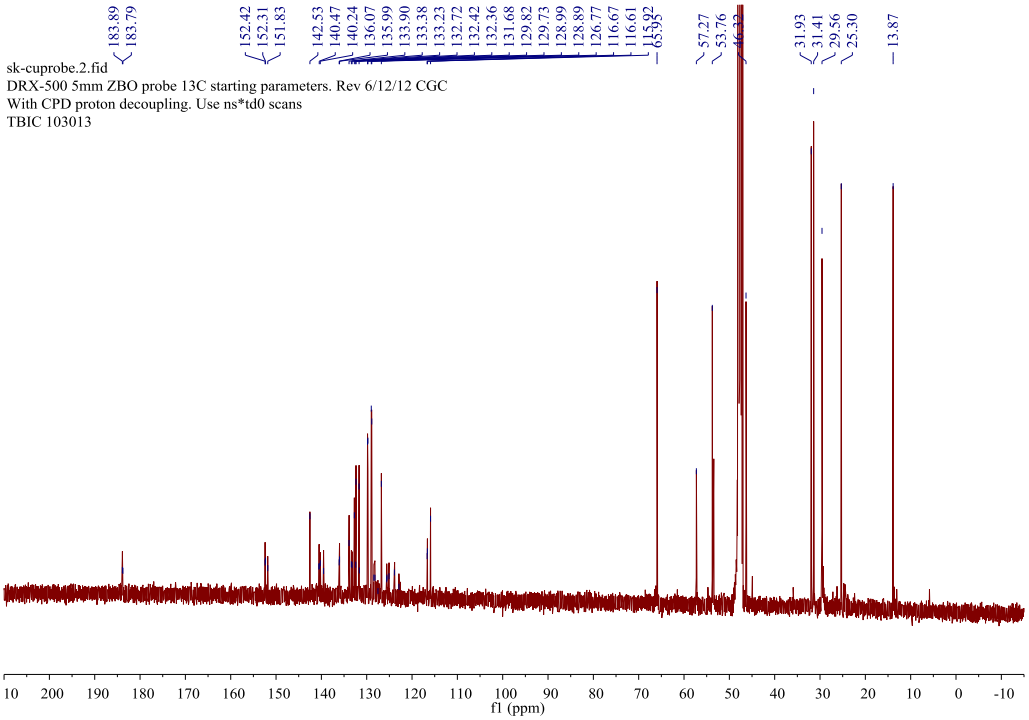
^{13}C NMR spectrum of Ctrl-CSF1 ($CDCl_3$, 101 MHz):



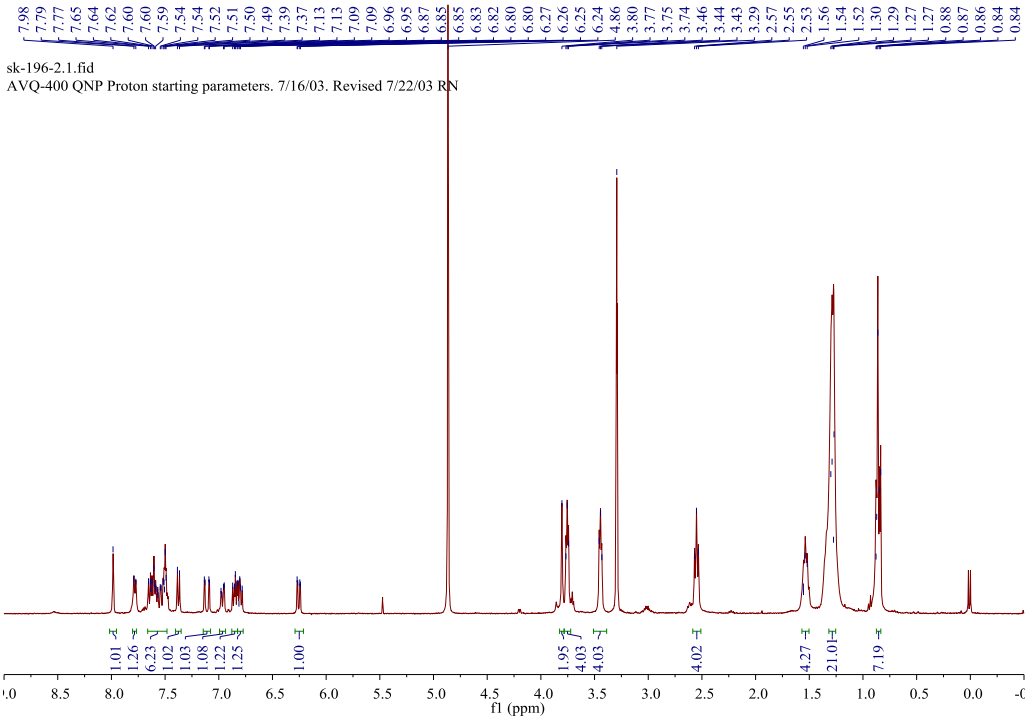
^{1}H NMR spectrum of CPF1 (CD_3OD, 400 MHz):



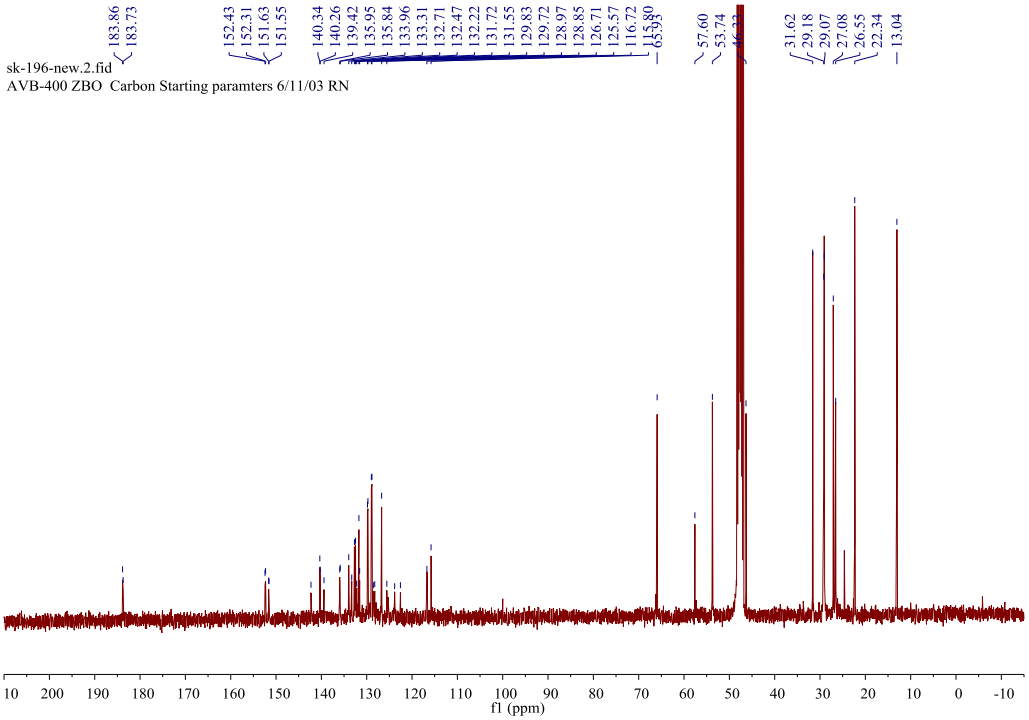
^{13}C NMR spectrum of CPF1 (CD_3OD, 125 MHz):



^{1}H NMR spectrum of Ctrl-CPF1 (CD_3OD, 400 MHz):



^{13}C NMR spectrum of Ctrl-CPF1 (CD_3OD, 101 MHz):



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